Pollination of cucumber, *Cucumis sativus* L. (Cucurbitales: Cucurbitaceae), by the stingless bees *Scaptotrigona* aff. *depilis* Moure and *Nannotrigona testaceicornis* Lepeletier (Hymenoptera: Meliponini) in greenhouses

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Polinização de Pepino, *Cucumis sativus* L. (Cucurbitales: Cucurbitaceae), pelas Abelhas sem Ferrão *Scaptotrigona* aff. *depilis* Moure e *Nannotrigona testaceicornis* Lepeletier (Hymenoptera: Meliponini) em Casas de Vegetação

**RESUMO** - Quando a fertilização de flores é necessária para o desenvolvimento de frutos, abelhas podem ser utilizadas como polinizadores sob cultivo protegido. No presente estudo, a efetividade das abelhas sem ferrão *Scaptotrigona* aff. *depilis* Moure e *Nannotrigona testaceicornis* Lepeletier como polinizadoras de pepino (*Cucumis sativus* var. caipira) foi investigada sob cultivo protegido durante o inverno. O estudo foi conduzido em quatro casas de vegetação (GH), das quais duas continham colônias de abelhas para averiguar a polinização dos pepinos (GH I, com *S*. aff. *depilis*, GH II, com *N. testaceicornis*), e duas (GH III, GH IV) não continham colônias e serviram como grupos controle. Além disso, pepinos foram plantados numa área aberta (AO) onde polinização por vários insetos poderia ocorrer. Sem polinização (GH III, GH IV), as plantas produziram menor número de pepinos, e os frutos eram menores e menos pesados do que aqueles nas áreas experimentais onde a polinização ocorreu. Na área aberta, não protegida contra condições climáticas desfavoráveis, as plantas produziram menos flores do que as plantas nas casas de vegetação. A maior produção de pepinos (com a maior quantidade de frutos perfeitos) foi encontrada nas casas de vegetação com as abelhas como polinizadoras (GH I, GH II). Os resultados demonstraram que abelhas sem ferrão podem ser usadas com sucesso e eficiência como polinizadoras de pepinos sob cultivo protegido durante o inverno.

**PALAVRAS-CHAVE:** Cultivo protegido, abelha social brasileira, produção de frutos

**ABSTRACT** - When for a successful fruit development the fertilization of flowers is necessary, bees can be used as crop-pollinators in greenhouses. In the present study, we investigated the effectiveness of the stingless bees *Scaptotrigona* aff. *depilis* Moure and *Nannotrigona testaceicornis* Lepeletier as pollinators of cucumber plants (*Cucumis sativus* var. caipira) in greenhouses during the Brazilian winter season. The study was conducted in four greenhouses (GH), of which two greenhouses contained bee colonies to ascertain pollination of the cucumber plants (GH I, with *S*. aff. *depilis*, GH II, with *N. testaceicornis*), whereas the other two greenhouses (GH III, GH IV) had no bee colonies and served as control groups. Furthermore, we planted cucumbers in an open field plot (OA) where pollination by any/Various visiting insects could occur. Each of the experimental areas measured 87.5 m². Without pollination (GH III, GH IV), the plants produced a low number of cucumbers, and the fruits were smaller and less heavy than in those experimental areas where pollination occurred. In the open field area, not protected against unfavorable climatic conditions, the plants produced fewer flowers than the plants in the greenhouses. The highest cucumber yield (with the highest amount of perfect fruits) was found in those greenhouses which housed the stingless bees as pollinators (GH I, GH II). Our results demonstrate that stingless bees can be successfully and efficiently used as pollinators of greenhouse cucumbers during the winter season.

**KEY WORDS:** Greenhouse crop, Brazilian social bee, fruit production

Most species of eusocial bees (honey bees, stingless bees and bumble bees) are visitors of flowering plants and are important pollinators of crops (McGregor 1976, Roubik 1995). The honeybee, *Apis mellifera* L., is considered the
principal pollinator of many crops due to several important traits, such as the high number of individuals per colony and its ability to recruit many workers to visit rich resources.

In tropical regions, however, stingless bees (Meliponini) are important pollinating agents of numerous native plant species (Roubik 1995). Many species of stingless bees contribute to the pollination of commercially important crops, including the coconut, macadamia nut, mango, mapati and anatto (Heard 1999). Recent studies demonstrate that stingless bees are also an effective alternative to honeybees for the pollination of many greenhouse crops of considerable economic and social importance, such as strawberries (Maeta et al. 1992, Kukutani et al. 1993, Malagodi-Braga & Kleinert 2004), tomatoes (Macias et al. 2001) and sweet peppers (Cruz et al. 2003). Even so, management techniques for these bees under greenhouse conditions have so far barely been investigated.

In recent years both production and consumption of cucumbers in Brazil, mainly of the caipira variety, have increased (Fnp Consultoria e Comércio 2000). In the State of São Paulo alone, 34,508 tons of cucumber were commercialized in 1998 (Cardoso 2002). Yet, the productivity of cucumbers cultivated in open field, about 1.6 kg/m^2, is still low in comparison to other crops like salad, which has a yield of 2.5 to 8.0 kg/m^2 (Santos 1980, Filgueira 1981). This poor harvest is due to the low temperatures during the winter season which damages many cucumber varieties. This damage, however, can largely be reduced by cultivating cucumbers in greenhouses (Cardoso 2002), which considerably increases the cucumber production to 15-30 kg/m^2 (Serrano Cermeño 1979, Robledo & Martin 1981, Alfonso Osório 1984). Contrary to the open field, in greenhouses the climatic conditions favorable for growing cucumber plants can easily be accomplished. These conditions comprise a high relative humidity (70-90%), soil temperature of 25-30°C and an ambient temperature which never drops below 12°C. The optimum temperature for fruit maturation is approximately 25-30°C (Serrano Cermeño 1979, Robledo & Martin 1981, Castilla 1990).

Most cucumber hybrids used as commercial crop are parthenocarpic, so they can produce fruits without fertilization (pollination). Consequently, these hybrids can be cultivated in greenhouses all year round without problem. One of Brazilian most important commercial cucumbers, however, the caipira variety, is not parthenocarpic. Hence, for a successful yield of this cucumber variety in greenhouses pollinating agents are indispensable (Godoy & Cardoso 2004).

In the present study, we investigated the effectiveness of two Brazilian stingless bee species, Scaptotrigona aff. depilis Moure and Nannotrigona testaceicornis Lepeletier, as pollinators of the ‘caipira’ cucumber in greenhouses during the Brazilian winter season. These bee species were chosen because they are among the most common stingless bees in the studied area and are widespread in several parts of the country including some of the coolest Brazilian locations. Their ample regional distribution, as well as the fact that their colonies can be easily maintained and multiplied, renders these two stingless bee species promising alternative to other commercially established pollinators, such as the honeybee.

### Material and Methods

#### Bees and plants utilized in this study.

Stingless bees (Apidae, Meliponini) are a group of mainly tropical, highly eusocial bees comprising about 400 species, distributed among more than 20 genera (Michener 2000). Bees of the genus Scaptotrigona are found from Rio Grande do Sul, Southern Brazil, all the way up to Mexico. Their colony population can reach up to 50,000 individuals (Nogueira-Neto 1970). Under disturbed conditions, e.g. when animals or people get close to the nest entrance, or when the nest is opened, the workers show an aggressive behavior during which many bees leave the nest, and attack and bite the “intruder” (Wille & Michener 1973). The body length of S. aff. depilis workers is 6-7 mm, their head and thorax are 2.6 mm, and 2.5 mm in width, respectively. The geographical distribution of the genus Nannotrigona ranges from the State of Parana in Southern Brazil to Mexico. Their colonies have populations between 2,000 and 3,000 adult individuals (Nogueira-Neto 1970, Michener 1974). In contrast to Scaptotrigona, the workers of Nannotrigona are not aggressive at all. Workers of N. testaceicornis have a body length of 5-6 mm, their head and thorax being 1.9 mm and 1.4 mm in width.

The cucumber (Cucumis sativus) is a trailing or climbing, normally monoeccious, annual herb, varying from 0.6 to 3 m in length. Its stem is covered with stiff, bristly hairs. Normal cucumber types have staminate and pistillate flowers in varying proportions depending on plant growth, vigor and environmental conditions. The fruit is pendulous and oblong and has a relatively large stem (McGregor 1976).

#### Experimental setup.

The present study was carried out in four greenhouses (GH I - GH IV), and in an external open area (OA), each measuring 87.5 m^2. The greenhouses and the open area were located at the campus of the Universidade de São Paulo, in Ribeirão Preto, São Paulo State, Brazil. The greenhouses had a transparent plastic roof and were laterally covered with a dark plastic screen, which reduced the luminosity by 30%.

*C. sativus* var. caipira was grown from seed. In both the greenhouses and the open area we planted 210 cucumber seedlings (30 days old) each. The cucumber plants were distributed in seven rows, spaced 30 cm apart, and they were artificially irrigated twice a day. We regularly observed the flower and fruit production from the day the first flower opened (May 15th, 2002) until the end of the study (July 26th, 2002). Our original objective, to count all flowers during the study to determine the proportion of flowers that actually turned into fruit, could not be accomplished because individually marking open flowers with a piece of thread destructed the flowers. However, it provided a good idea about whether a similar amount of flowers was produced in all experimental areas. Therefore, we only exemplarily registered the number of open male and female flowers on four days (May 30th, June 9th, June 18th, and June 23rd). The number of open flowers was counted at 11 a.m. because early in the morning not all cucumber flowers had opened.

The first greenhouse (GH I) contained two colonies of *S. aff.* depilis, with about 2,500 individuals each. The second
greenhouse (GH II) had four colonies of *N. testaceicornis*, with about 1,500 individuals each. So the number of individuals was similar in both greenhouses. The two remaining greenhouses (GH III and GH IV) contained no bee colonies and served as control groups. The field area outside the greenhouses (OA) was open to all potential pollinators, and also to non-pollinators that visited the plants. We collected about one specimen per flower-visiting species with an entomological net and placed them in a small glass bottle for further identification. In all experimental areas, we measured the temperature and the relative humidity at 8 a.m., 10 a.m., 11 a.m., 2 p.m. and 4 p.m.

**Flower visits.** During 20 min per hour, from 9 a.m. to 4 p.m. (the main period of bee activity), we recorded all flower visits by bees in the greenhouses GH I and GH II. Both stingless bee species used in our experiments did not utilize the cucumber flowers as pollen resource. However, nectar collection was frequently observed. In order to investigate whether both bee species were actually able to pollinate the flowers during their nectar collection, we recorded the bees’ behavior through direct observation, photographs and with a video camera. Observations on the bees’ behavior were performed from June 5th to June 25th, 2002.

**Fruit production and measurements.** To determine the crop yield of each experimental plot, the four greenhouses and the open area, respectively, we counted the total number of fruits produced therein. An aleatory sample of 100 fresh cucumbers was collected from each plot. In one of the greenhouses, GH IV, only 28 fruits were produced during the entire study. These were all included into the analysis. The fruits were weighed to the nearest 0.1 g, and their length, as well as their basal, their apical and their equatorial girth were determined (Fig. 1).

To determine these size parameters, we used a piece of thread which was marked with a pen at the respective position during the measurement and afterwards measured with a common ruler to the nearest 0.5 cm. Fruits that presented size variations (too large or too small when compared to 10 commercially available cucumbers), a twisted shape, or a yellowish color were considered imperfect. Both the production of seeds and the seed weight are important parameters for the future reproductive success of a plant (Primack 1987).

To get an insight into this fruit feature, 10 seeds (if existent) were taken out of a sample of 30 randomly chosen fruits from each plot. The seeds were naturally dried and weighed to the nearest milligram.

**Statistics.** The mean numbers of male and female flowers in each experimental area were compared using the Student-t test. The Chi-squared test was applied to compare the proportion of perfect/imperfect fruits between the different plots. The Kruskal-Wallis test was used to compare the different fruit quality parameters among the different experimental areas (post-hoc pairwise comparison: Dunn’s test). The level of significance of differences was taken as $P \leq 0.05$. When data sets were used for more than one statistical comparison (in case of the Chi-squared tests), a Bonferroni correction for the level of significance was performed ($P_{corr.} \leq 0.05/number$ of comparisons) (Sokal & Rohlf 1995). If not specified otherwise, values are presented as mean ± 1SD. The statistical analysis was performed using the software SigmaStat 3.1. All graphs were created with the software SigmaPlot 2001.

**Results**

**Flower production.** The cucumber flowers started to open at 8 a.m. and by 11:30 a.m. usually all of them had opened. The closing of the flowers occurred around 1:30 p.m. The flowers lasted for between one and four days before withering. Because individually marking the flowers considerably damaged them (see above), we only exemplarily counted the number of open male and female flowers on four days during the study. In all greenhouses, male and female flowers were produced in similar proportions (Student t-test, $P > 0.05$; Fig. 2). Only in the open field plot, we counted significantly more male than female flowers (Student t-test, $t = 3.98$, df = 6, $P = 0.007$). Considering the total amount of flowers, we registered more than 200 open flowers in the greenhouses and less than 100 open flowers in the open area per count (total open flowers: GH I, 244.3 ± 59.4; GH II, 259.8 ± 110.9; GH III, 352.3 ± 73.5; GH IV, 210.0 ± 97.2; OA, 98.3 ± 17.9).

**Flower visits.** In both experimental greenhouses (GH I, GH II), both male and female flowers were regularly visited by the stingless bees. Although neither *S. aff. depilis* nor *N. testaceicornis* collected pollen on purpose during their visits, the pollen grains from the anthers were adhered to the hairs of the bees’ heads and bodies during the nectar uptake. Hence, pollen transfer between flowers and, in consequence, fertilization of the female cucumber flowers certainly occurred in the greenhouses with the bee colonies. During the observation period (June 5th - June 25th), we registered the first bees collecting nectar from cucumber flowers at 9 a.m.

![Fig. 1. Size measurements of cucumber fruits. The basal (BG) and apical (AG) girths were measured just prior to apical and basal curvature. The equatorial girth (EG) was measured in the exact central region of the fruit. The length (L) was defined as the entire extent of the cucumber, from the point of basal to the apical curvature.](image-url)
The study. Asterisk indicates significant differences between the different experimental areas. Bars represent the means (+1SD) of four measurements made during the study. Asterisk indicates significant difference between the number of male and female flowers (Student-t test: P < 0.05).

Following bee species were found on the cucumber flowers in the open field plot (OA): A. mellifera; Paratrigona lineata (Apidae: Meliponini); Dialectus (Chloralictus) sp. (Halictidae); Exomalopsis sp. (Anthophoridae). Furthermore, we found beetles of the families Chrysomelidae and Galericidae on the cucumber flowers in the open area.

Fruit production. We measured a very similar ambient temperature and relative humidity in the four greenhouses, as well as in the open field area (temperature: GH I, 26.7 ± 3.6°C; GH II, 26.4 ± 4.3°C; GH III, 25.6°C ± 3.5°C; GH IV, 25.8 ± 3.8°C; OA, 25.5 ± 3.9°C; relative humidity: GH I, 41.7 ± 9.8; GH II, 41.2 ± 9.2%; GH III 42.2 ± 10.1%; GH IV 40.0 ± 10.4%; OA, 42.0 ± 9.9%)

Fruits of pollinated versus fruits of not-pollinated cucumber plants in greenhouses - In the four greenhouses, the cucumber plants produced a similar amount of flowers (see above). However, there were significant differences between the greenhouses with bee colonies (GH I, GH II) and those without pollinators (GH III, GH IV) in both quantity and quality of fruits produced therein. Whereas in both greenhouses containing bee colonies the yield was more than 400 cucumbers, in the greenhouses without pollinators only 276 fruits (GH III) and 28 fruits (GH IV) matured (Table 1). The proportion of imperfect fruits was higher in those greenhouses where flowers had not been pollinated by bees than in those that housed the bee colonies (Chi-square test: GH I x GH III, $\chi^2 = 24.4$, P < 0.001; GH II x GH III, $\chi^2 = 36.9$, P < 0.001; Table 1)

Concerning the quality parameters, cucumbers collected in GH I (with S. aff. depilis) and GH II (N. testaceicornis) were significantly larger and heavier than those collected in GH III and GH IV (no bees) (Dunn’s pairwise comparison: P < 0.05; Fig. 4). Moreover, the fruits from the greenhouses without bee pollination did not produce seeds (Fig. 4).

Fruits of pollinated plants in greenhouses versus fruits of pollinated plants in the field - In the open field (OA), less cucumbers were produced than in the greenhouses that housed bee colonies (GH I, GH II) (Table 1). This finding is possibly due to the smaller amount of flowers produced in the open field plot (see above). Most fruit quality parameters,

Table 1. Cucumber (C. sativus) yield in the different experimental areas exposed to stingless bees colonies (GH I, GH II), no-bees (GH III, GH IV) and in open field plot (OA).

<table>
<thead>
<tr>
<th></th>
<th>No. of produced cucumbers</th>
<th>Imperfect fruits</th>
</tr>
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<tbody>
<tr>
<td>Grenhouse I (GH I)</td>
<td>409</td>
<td>25</td>
</tr>
<tr>
<td>Grenhouse II (GH II)</td>
<td>413</td>
<td>17</td>
</tr>
<tr>
<td>Grenhouse III (GH III)</td>
<td>276</td>
<td>50</td>
</tr>
<tr>
<td>Grenhouse IV (GH IV)</td>
<td>28</td>
<td>3</td>
</tr>
<tr>
<td>Open field plot (OA)</td>
<td>321</td>
<td>42</td>
</tr>
</tbody>
</table>
Fig. 4. Quality of cucumbers collected in the different experimental areas. The parameters taken for quality were (a) basal (BG), equatorial (EG), apical girth (AG), and length (L) of the fruits, as well as (b) their weight, and (c) weight of their seeds. Bars represent the means (+1SD) of the measurements (for GH I-III and OA, fruit size and weight, N = 100; seed weight, N = 30; GH IV, N = 28). Same letters above bars indicate no significant differences between the experimental areas (Dunn’s pairwise comparison: P > 0.05). For explanation of bar-shading see Fig. 2. Note: cucumbers in greenhouses GH III and GH IV had no seeds.

Fruits of plants pollinated by stingless bees in greenhouses - Neither in the amount of fruits produced nor in any of the measured quality parameters were found significant differences between the fruits deriving from flowers pollinated by S. aff. depilis and those deriving from flowers pollinated by N. testaceicornis (fruit weight and size, seed weight: Dunn’s pairwise comparison: P > 0.05; proportion perfect/imperfect fruits: Chi-square test: P > 0.0125; Fig. 4, Table 1).

Discussion

Cucumbers present an economically important crop in Brazil (Cardoso 2002). Unfortunately, climatic conditions during the winter, like low ambient temperature and low relative humidity, dramatically diminish cucumber yield. Climatic protection in greenhouses is a reasonable solution only for cucumber hybrids that are parthenocarpic and, therefore, don’t need fertilization by pollinators. In the present study, we demonstrate that also the yield of the ‘caipira’ cucumber, which is not parthenocarpic, can be successfully increased by cultivating it in greenhouses, but only if pollinating agents are present (Fig. 5). Fruits in the greenhouses without pollinators (GH III, GH IV) could only derive from self- or wind-pollinated flowers. In these control greenhouses, only a small number of fruits were produced (Table 1). But not only was the fruit quantity diminished when pollinating agents were absent but also the fruit quality (Fig. 4, Table 1).

So far, little is known about the effects of bee pollination...
on the cucumber production. Commonly, *C. sativus* flowers are visited by halictid bees. In North America, the most effective pollinator of cucumber flowers is the solitary bee *Melissodes communis* (Free 1993). Studies on the cucumber species *C. sativus* and *C. melo*, showed that the honeybee, *A. mellifera*, could be an efficient pollinating agent for commercial crops. Fruits deriving from flowers which had been pollinated by honeybees had a better quality than those deriving from flowers without bee pollination (for *C. sativus*: Couto & Calmona 1993) from manually pollinated flowers (for *C. melo*: Marchini et al. 2006). However, *A. mellifera* usually visit cucumber flowers only when no other, more attractive flowers are present.

Both species of stingless bees used in the present study, *S. aff. depilis* and *N. testaceicornis*, are certainly promising candidates for the pollination of cucumber flowers in greenhouses, at least in Neotropical regions. Like most species of stingless bees, both *S. aff. depilis* and *N. testaceicornis* show a high floral constancy (*S. aff. depilis*: Bego, unpublished; *N. testaceicornis*: Bego et al. 1989) and easily adapt to new plant species (Heard 1999). These features are important traits for the potential application of these bees as pollinators of greenhouse crops. Also, the peak activity period of the foragers (Carvalho & Bego 1995; Fig. 3) fits well to the flowering peak of the cucumber flowers (approximately 10 a.m. - 1 p.m.). Most importantly, however, both used bee species are well adapted to relative low ambient temperatures because they inhabit even the coolest regions of Brazil. Hence, it is possible that they can be used as pollinators of greenhouse crops in some places during the Brazilian winter period, as we demonstrated with the present study.

The winter period is climatically the most critical time for the cucumber cultivation because ambient temperatures of between 25°C and 30°C (never below 12°C) and a high relative humidity (70-90%) are required for a successful growing of the crop (Martins et al. 1995). Although the mean daytime temperatures measured during the study were within the range which is optimal for the cucumber plants, the nighttime temperatures were around 14°C, and even dropped to 11°C at an early stage of the flowering (May 27th) (source: http://www.ciaagro.sp.gov.br). This certainly influenced the flowering and the growing of the crop because the ‘caipira’ cucumber does not adapt well to low temperatures (Cardoso & Silva 2003). Within the greenhouses, however, the plants were at least partly protected from the unfavorable climatic conditions. This becomes obvious from the fact that in all four greenhouses the cucumber plants produced more flowers than the plants grown in the open field area (Fig. 2). Also, the vegetative parts of the plants suffered less damage through the low temperature in the greenhouses than in the open field (S. A. Bispo dos Santos, pers. observation). Maybe even the higher proportion of imperfect fruits in the open field compared to fruits from the greenhouses with the pollinators (Table 1) can be blamed on the missing climatic protection.

Combining the two conditions, bee pollination and climatic protection, we should expect a considerable increase in the ‘caipira’ cucumber yield in those greenhouses which housed the stingless bee colonies. And indeed, calculating the cucumber yield (kg/m²) from the data of our study, we find the highest outcome in the two greenhouses with the bee pollinators (Fig. 5). The yield of all fruits, perfect and imperfect ones, in the greenhouses with *S. aff. depilis* (1.37 kg/m²) and *N. testaceicornis* (1.53 kg/m²) is 21.6% and 35.4% higher than the cucumber yield of the open area (1.16 kg/m²). Taking only commercially suitable fruit (“perfect” cucumbers) into account, the outcome of the two bee-greenhouses is even better due to the small proportion of imperfect fruits produced therein (Table 1). The yield of perfect cucumbers is increased by 27.3% (GH I, *S. aff. depilis*, 1.29 kg/m²) and by 45.5% (GH II, *N. testaceicornis*, 1.47 kg/m²) when compared to the perfect fruit yield of the open area (OA, 1.01 kg/m²) (Fig. 5).

Cucumber pollination by stingless bees (*S. aff. depilis* and *N. testaceicornis*) can increase the quantity and quality of fruits production.

**Acknowledgments**

We are grateful to Dr. David De Jong and Professor Andrew Alastair Cumming for linguistic revision; Dr. Breno Magalhães Freitas for his guidance at the beginning of the experimental period; FAPESP, CAPES and CNPq for financial support.

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Received 26/IX/06. Accepted 22/V/08.